Cytotherapy, 2015; 17: 1280–1291

International Society for Cellular Therapy
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T cells specific for different latent and lytic viral proteins efficiently control Epstein-Barr virus–transformed B cells

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Abstract

Background aims. Epstein-Barr virus (EBV)-associated post-transplant lymphoproliferative disorders (PTLD) belong to the most dreaded complications of immunosuppression. The efficacy of EBV-specific T-cell transfer for PTLD has been previously shown, yet the optimal choice of EBV-derived antigens inducing polyclonal $CD4^+$ and $CD8^+$ T cells that cover a wide range of human leukocyte antigen types and efficiently control PTLD remains unclear. *Methods.* A pool of 125 T-cell epitopes from seven latent and nine lytic EBV-derived proteins (EBV_{mix}) and peptide pools of EBNA1, EBNA3c, LMP2a and BZLF1 were used to determine T-cell frequencies and to isolate T cells through the use of the interferon (IFN)-γ cytokine capture system. We further evaluated the phenotype and functionality of the generated T-cell lines *in vitro*. *Results.* EBV_{mix} induced significantly higher T-cell frequencies and allowed selecting more $CD4^+IFN\text{-}\gamma^+$ and $CD8^+IFN\text{-}\gamma^+$ cells than single peptide pools. T cells of all specificities expanded similarly *in vitro*, recognized cognate antigen, and, to a lower extent, EBV-infected cells, exerted moderate cytotoxicity and showed reduced alloreactivity. However, EBV_{mix}-specific cells most efficiently controlled EBV-infected lymphoblastoid cell lines (LCLs). This control was mainly mediated by EBV-specific $CD8^+$ cells with an oligoclonal epitope signature covering both latent and lytic viral proteins. Notably, EBV-specific $CD4^+$ cells unable to control LCLs produced significantly less perforin and granzyme B, probably because of limited LCL epitope presentation. *Conclusions.* EBV_{mix} induces a broader T-cell response, probably because of its coverage of latent and lytic EBV-derived proteins that may be important to control EBV-transformed B cells and might offer an improvement of T-cell therapies.

Key Words: *T-cell transfer, Epstein-Barr virus, IFN-γ selection, immunotherapy, PTLD*

Introduction

Epstein-Barr virus (EBV)-associated post-transplant lymphoproliferative diseases (PTLD) cause significant mortality after transplantation [1–3]. Immunosuppression reduces the number and/or function of EBV-specific T cells, resulting in uncontrolled proliferation of EBV-infected B cells and tumor formation [1–5]. Treatment strategies include withdrawal of immunosuppression, anti-CD20 antibodies that eliminate B cells—the main reservoir of EBV—or sequential immunochemotherapy with anti-CD20 antibodies and cyclophosphamide, doxorubicin, vincristine and prednisolone (CHOP) chemotherapy [6,7]. Nevertheless, these treatments fail in up to 60% of cases [7].

Transfer of functional EBV-specific T cells may control PTLD after transplantation when the recipient's adaptive immunity is not yet restored. It was previously shown that adoptive transfer of donor-derived EBV-specific T cells is safe and can efficiently control PTLD [8–12]. Particularly, recent studies that use a rapid expansion protocol or

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(Received 3 April 2015; accepted 7 June 2015)

ISSN 1465-3249
http://dx.doi.org/10.1016/j.jcyt.2015.06.003

immunomagnetic sorting with the interferon (IFN)-γ cytokine capture system (CCS) for direct infusion are promising [8,9,11]. However, it is not clear yet, which EBV epitopes most efficiently induce polyclonal $CD4^+$ and $CD8^+$ T cells that control PTLD and cover a wide range of human leukocyte antigen (HLA) types [8,9,13,14]. Moreover, the function and necessity of EBV-specific $CD4^+$ cells to control PTLD is debated, especially in relation to their cytotoxic potential [10,12,15–18].

PTLDs express latent viral proteins, i.e., EBV nuclear antigens (EBNA1, 2 and 3 and EBNA-LP) and latent membrane proteins (LMP1 and LMP2), but also several lytic proteins, for example, *Bam* HI Z restriction fragment of EBV, beginning with the Leftward OR Frame number 1 (BZLF1) [4,19]. Because there are no reliable animal models of PTLD [20], *in vitro*–generated EBV-infected B cells (lymphoblastoid cell lines [LCL]) that express a similar viral gene profile are currently the best model to study PTLDs and PTLD-directed immune responses [5,21].

In the present study, we compared a pool of $CD8^+$ and $CD4^+$ T-cell epitopes from seven latent and nine lytic EBV-derived proteins (EBV_{mix}) [22] to commercially available LMP2a, EBNA1, EBNA3c and BZLF1 peptide pools. We assessed the EBV-specific T-cell frequencies in healthy donors and hematopoietic stem cell transplant (HSCT) recipients. With the use of the IFN-γ CCS, we isolated EBV-specific cells and evaluated the $CD4^+$ and $CD8^+$ T-cell specificity and their short- and long-term functionality against LCLs.

Methods

EBV-derived peptide pools

Commercially available peptide pools covering complete sequences of EBNA1, LMP2a, BZLF1 (PepTivator, Miltenyi Biotec) and EBNA3c (JPT Peptide Technologies) consist of 15-mer peptides overlapping by 11 amino acids. The pool of 91 HLA class I and 34 HLA class II T-cell epitopes of 8 to 20-mers derived from 16 latent and lytic proteins (EBV_{mix}) was provided by C.H. and C.B. (Supplementary Table S1) [22].

Patients and healthy donors, cell isolation and generation of dendritic cells and LCLs

Blood was obtained from adult healthy donors and adult HSCT recipients after written informed consent and approval by the Ethical Committee Nordwest-und Zentralschweiz, Switzerland (No. 242/11). Healthy donors were HLA-typed (donor 3: A1, A11, B08, B35, C04, C07, DR1, DR17, DQ02 and DQ05; donor 4: A11, A24, B07, C07, DRB1 15, DRB5 01 and DRB6 02; donor 6: A02, A203, B13, B44, C05, C06, DR07, DR16, DQ02 and DQ05; donor 9: A02, A203, A23, B44, B55, B22, C03, C04, DR07, DR13, DR14, DQ02 and DQ06; donor 13: A01, A23, B08, B44, DRB1 03, DRB1 07 and DQB1 02). HSCT recipients were in median 54 months (range, 30–108) after HSCT, under persistent immunosuppression and replicated EBV in the blood 2 to 10 months before blood sampling. EBV–polymerase chain reaction was performed in whole blood as previously published [23]. The study was conducted according to the Declaration of Helsinki. Isolation of peripheral blood mononuclear cells (PBMCs) and generation of dendritic cells (DCs) were performed as previously published [24]. LCLs were generated from autologous PBMCs infected with B95.8 EBV [20].

IFN-γ–based CCS and expansion of T cells

After 4-h stimulation of PBMCs (1×10^8) with respective peptide pools, EBV-specific cells were enriched with the use of the Large Scale IFN-γ Secretion Assay-Enrichment Kit (Miltenyi Biotec) according to the manufacturer's instruction. Unstimulated PBMC served as control. The positive cell fractions were investigated with anti–IFN-γ–PE (Miltenyi Biotec), anti–CD4-PacificBlue and anti–CD8-APC (all BioLegend) staining with the use of a CyAn ADP Flow Cytometer (DAKO Cytomation) and analyzed with the use of FlowJo software (Tree Star). The isolated cells were then expanded to increase their numbers for further characterization *in vitro*. Up to 4×10^5 cells from the IFN-γ–positive fraction were expanded with 1:50 γ-irradiated (35 Gy) autologous feeder cells [25]. Cultures were supplemented with 5 U/mL interleukin (IL)-2 (Proleukin, Novartis) until day 7 and additionally with 10 ng/mL IL-7 and IL-15 (PeproTech) from day 7 to day 14 [25]. Thereafter, specificity was assessed by means of intracellular cytokine (ICC) staining, and cells were cryopreserved. The T-cell lines were expanded for further analysis as previously published [26]. $CD4^+$ and $CD8^+$ T cells were selected from T-cell lines with the use of CD8 or CD4 MicroBeads (Miltenyi Biotec) according to the manufacturer's instruction to at least 95% purity and used immediately or expanded by the rapid expansion protocol.

Enzyme-linked immunospot assay and ICC staining

Frequencies of cytokine-producing cells in PBMCs and in T-cell and CD4-lines were assessed by means